## PS176.

### Rescuing Effect of Autologous Mesenchymal Stem Cells in a Model of Early Type II Diabetes

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**Objectives:** Repeated, low dose injections of Streptozotocin (STZ) create a model of Type II Diabetes Mellitus. We utilize this model to investigate the potential pro-repair effect of syngeneic (equivalent to autologous) bone marrow-derived mesenchymal stem cells (BMD-MSC) in Diabetes.

**Methods:** STZ (40 mg/Kg) was administered, daily, for five days, i.p., in C57BL6 mice (N = 12). Weekly blood glucose levels (BG) were monitored by 2-hour Glucose Tolerance Test (GTT) for five weeks after. Glucose intolerance (150 < BG < 200 mg/dL) was used as the trigger for treatment. The mice were then divided in two groups (N = 6/group). Control group received PBS and treatment group received MSC (1x10-6), by tail vein infusion. MSC were obtained by culturing BMD cells from donor mice (N = 3) in MesenCult® medium. β-cell activity was measured by immunohistochemistry, and serum level of insulin was assessed by C-peptide test.

**Results:** At baseline and post-STZ-induction, GTT between control and treatment group did not differ significantly. However, at week 3 the MSC treatment group's GTT normalized and the effect is sustained by week 5 (Fig). Control group continues to have significant glucose intolerance. Longer follow up is ongoing.

**Conclusions:** Systemic injection of a single MSC treatment improves the Glucose Tolerance in a murine model of Type II Diabetes. The findings will require confirmation in genetic murine models, yet depict a highly promising novel concept for the treatment of Diabetes.

**Author Disclosures: Z. Liu**: Nothing to disclose; **A. Livingstone**: Nothing to disclose; **R. Tian**: Nothing to disclose; **O. C. Velazquez**: Nothing to disclose; **B. Wang**: Nothing to disclose.

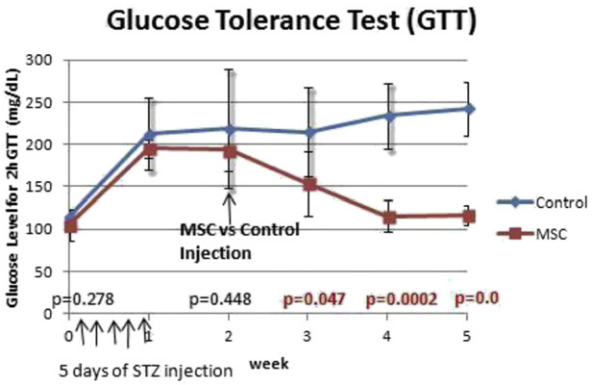


**Fig.**

## PS178.

### Supplemental Oxygen Reverses Hypoxia Induced Smooth Muscle Cell Proliferation by Modulating HIF-alpha and VEGF Levels in a Rabbit Arteriovenous Fistula Model

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**Objectives:** Numerous mechanisms for the formation of intimal hyperplasia have been proposed but none have been proven or accepted. Our research focuses on the potential role of Hypoxia Inducible Factors (HIFs), VEGF, and PDGF as well as the ERK, PI3-K/AKT pathway in hypoxia mediated intimal hyperplasia processes.

**Methods:** Rabbits were randomized into different experimental groups with varying oxygen exposure and receipt of surgery. Plasma samples were collected at designated intervals in which cytokines and smooth muscle cell proliferation were measured. In addition, cell specimens were exposed to hyperoxic, normoxic, and hypoxic environments with cytokines measured at various time points.

**Results:** Placement of an arteriovenous fistula resulted in hypoxia induced HIF stabilization with a concurrent increase in VEGF. Activation of VEGF receptors on smooth muscle cells through ERK1 and AKT pathways resulted in significant smooth muscle cell proliferation and migration. These effects are dramatically reduced in animals that are exposed to a hyperoxic environment of 30% oxygen.

**Conclusions:** Our results suggest that short-term administration of supplemental oxygen inhibits HIFs and VEGF signaling to reduce smooth muscle proliferation in the local blood vessel. These results provide strong support for the therapeutic use of supplemental oxygen following arterial surgery to reduce intimal hyperplasia. These findings also provide a nidus for future studies into the mechanisms of hypoxia induced intimal hyperplasia.

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## PS180.

### The Gene Expression of Adenosine Receptors in the Processes of Contrast Induced Nephropathy in Mouse Kidney

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**Objectives:** Contrast Induced Nephropathy (CIN) is the third leading cause of hospital acquired renal failure, which is a common complication following endovascular surgery procedures. The mechanism of CIN is not fully understood. Adenosine receptors (AR) regulate various physiological activities in kidney. We hypothesized that adenosine and its receptors may play a role in development of CIN. The objectives of this study were to investigate the expression changes of the four subtypes of adenosine receptors (A1AR, A2AAR, A2BAR, and A3AR) following administration of contrast agent in mice.

**Methods:** C57B1/6J mice were randomized to treatment and control groups. Iodixanol, a commonly used contrast agent, was administered to treatment groups

through retro-orbital injection at two different dosages, 0.75gI/kg and 2.75gI/kg. PBS was given to the mice in control group. Mice kidneys were harvested at day 3 and day 7 following Iodixanol administration. RNA and protein were then extracted. qRT -PCR and Western blot were used to quantify A1AR, A2AAR, A2BAR, and A3AR RNA and protein expression respectively, with GAPDH as endogenous control.

**Results:** qRT-PCR showed that Iodixanol induced AR transcription, specifically in the group treated with 2.75gI/kg at day 3 after injection. The RNA levels in all the four subtypes of ARs were increased 2-3 folds at day 3, but returned to normal at day 7 in Iodixanol groups compared to PBS controls. The Western blot results showed that A1AR, A2AAR, A3AR expressions were increased 1.5-2 folds in Iodixanol group at day 3 compare to PBS control. A2BAR expression was very low in normal physiological condition and no significant changes were detected by western blot.

**Conclusions:** Our results indicate that Iodixanol induces adenosine receptor gene expression in mice. Adenosine receptors may play a role in the development of CIN. Further investigation on the correlation between adenosine receptor gene expression and severity of CIN will be preformed.

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## C6k: Poster Session - Research (2)

### PS182.

### Inhibition of Angiogenesis and Endothelial Cell Function by WISP-1/CCN4

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**Objectives:** Wnt-induced secreted protein-1 (WISP-1/CCN4) is involved in regulating cell proliferation, survival/apoptosis, migration and tumor growth. We previously observed that tumor stroma-produced WISP-1/CCN4 plays a critical role in melanoma growth and tumor angiogenesis. We sought to determine the biologic effect of WISP-1/CCN4 on endothelial cell biology and angiogenesis.

**Methods:** The effects of WISP-1/CCN4 on cell growth and migration of human endothelial cells (EC) were examined by MTT and transwell-migration assay. Two- and three-dimensional (2D and 3D) angiogenesis models were used to study the role of WISP-1/CCN4 in vascular network formation of EC. Melanoma xenograft murine model was employed to examine the biological effect of WISP-1/CCN4 on tumor angiogenesis and growth. Human melanoma cells transduced with WISP-1/lentivirus or GFP/lentivirus were xenografted on skin of SCID mice (n = 6/group). Expression of exogenous WISP-1/CCN4 in transduced melanoma cells was validated by Western blot. Tumor angiogenesis was assessed by mouse whole-body Dil-perfusion and tumor tissue confocal microscopy. Tumor size was measured to determine tumor growth.

**Results:** WISP-1/CCN4 inhibited cell growth and migration of EC in vitro. Vascular network formation of EC in 2D- and 3D angiogenesis models was suppressed considerably ($P < .01$) by supplementation of γhWISP-1 (100 ng/mL) in both Matrigel (2D) and type I collagen gel (3D). Elevated expression of WISP-1 in tumor tissue significantly antagonized tumor angiogenesis and retarded tumor growth in vivo.

**Conclusions:** Our results revealed a novel role of WISP-1/CCN4 in negatively regulating endothelial cell biology and angiogenesis, implicating that WISP-1/CCN4 may serve as a novel target for cancer therapeutic intervention.

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### PS184.

### Differential Proliferative and Migratory Activity of Human Myoblasts Isolated from CLI and Control Patients

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**Objectives:** Patients with critical limb ischemia (CLI) have attributable myopathy that impairs their functional ability as well as dampening the positive effect of successful revascularization outcomes. Adult skeletal muscle retains the potential to repair following different injury patterns. We aim to investigate the potential differences in proliferation and migration capability of human myoblasts with CLI and asymptomatic control patients.

**Methods:** Gastrocnemius muscle biopsies were obtained from patients with critical limb ischemia and control samples were obtained from non-ischemic patients. Human myoblasts were isolated and cultured from each sample and stained with the myoblast marker desmin. Confirmed myoblast cultures (<5% alternative cell type contamination) were then used to investigate the proliferative capability (MTT assay) as well as migratory potential (scratch-wound assay) under both ischemic and normoxic conditions of each group.

**Results:** Myoblasts isolated from CLI patients demonstrated greater proliferative ability in comparison to control samples. However, control samples revealed greater capacity to heal scratch wounds made in a monolayer of myoblasts in both normoxia and hypoxic conditions.

**Conclusions:** Isolation of human myoblasts, especially from disease groups provides a useful platform to perform in vitro analysis to better understand and characterise pathology. CLI myoblasts exhibited enhanced proliferative but diminished migratory potential in comparison to control myoblasts.

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